REMARKS

Claims 1-127 are pending in the application. Claims 1-11, 14-40 and 53-89 are under consideration in the Office Action. Claims 12, 13, 41-52 and 90-127 are withdrawn from consideration as being directed to non-elected subject matter. Applicants hereby reserve the right to pursue the subject matter of the canceled claims in one or more divisional patent applications.

Claim Rejections Under 35 U.S.C. §112

Claims 1-11, 14-40, and 53-89 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The Examiner asserts that the claims are indefinite for recitation of the phrases "first portion" and "second portion." Applicants respectfully traverse.

Applicants describe the metes and bounds of a "portion." On page 5, lines 3-12, Applicants describe the method for producing and screening of cyclic peptide libraries *in vivo*:

> A general method for the *in vivo* production and screening of cyclic peptide libraries has been discovered. In this method, a nucleic acid molecule is constructed such that a nucleotide sequence encoding the peptide to be cyclized is flanked on one end with a nucleotide sequence encoding the **carboxy-terminal portion** of a split (or trans) intein (C-intein or I_C) and on its other end with a nucleotide sequence encoding the **amino-terminal portion** of a split intein (N-intein or I_N). Expression of the construct within a host system such as a bacterium or eukaryotic cell results in the production of a fusion protein. The two split intein components (i.e., I_C and I_N) of the fusion protein then assemble to form an active enzyme that splices the amino and carboxy termini together to generate a backbone cyclic peptide. (Emphasis added).

Applicants teach that a first portion and a second portion is an N-terminal portion of a split intein and a carboxy-terminal portion of a split intein wherein each portion alone has no activity and requires the assembly of these portions to form an active enzyme. The number of amino acids that comprise each portion is not important, but the fact that assembly of each portion must form an **active** enzyme. Applicants further teach examples of split inteins. See, for example, page 6, lines 16-19:

> Both the **first portion** of a split intein and the **second portion** of a split intein can be derived from a naturally-occurring split intein such as Ssp DnaE. In other variations, one or both of split intein portions can be derived from non-naturally occurring split inteins such as those derived from RecA, DnaB, Psp Pol-I, and Pfu inteins. (Emphasis added).

Applicants teach that the N- and C-terminal inteins are proteins that actually associate to form a complex that initiates and drives the cyclization reaction. (See for example, page 18, lines 5-23 through to page 2, lines 1-12 and figures 1 and 2). Within this complex the cyclization reaction occurs with the concomitant loss of the N- and C-terminal inteins. Applicants also teach that the portions of split inteins can be naturally occurring or artificially produced. See, for example, page 20, lines 11-24 through to page 21, lines 1-13:

> Nucleotide sequences that encode the **first portion** of a split intein and the **second portion** of a split intein of the nucleic acid molecules within the invention can be derived from **known inteins.** A fairly comprehensive and descriptive list of such inteins is published by New England Biolabs at http//www.neb.conljinteins/ifltreghtml Any of these known inteins can be used as long as they are compatible with invention.
>
> Nucleotide sequences that encode either naturally-occurring or artificially-produced split inteins can be used to generate the intein portions of nucleic acid molecules within the invention. Naturally-occurring split inteins are expressed in nature as two separate components that bind one another to form one active splicing agent. The nucleic acid molecules encoding these naturally-occurring components can thus be used in the invention. One example of a naturally-occurring split intein that may be used is Ssp DnaE (Wu et al, Proc. Natl. Acad. Sci. USA 95:9226,1998).

> Inteins that are not split in their natural state (i.e., those that exist as one continuous chain of amino acids) can be artificially split using known techniques. For example, two or more nucleic acid molecules encoding different portions of such inteins can be made so that their expression yields two or more artificially split intein components. See, e.g., Evans *et al. J. Biol. Chem.* 274:18359, 1999; Mills *et al, Proc. Natl. Acad. Sci.* USA 95:3543, 1998. The nucleic acids that encode such non-naturally occurring intein **components (portions)** can be used in the invention. Those nucleic acid molecules that encode non-naturally occurring split intein portions which efficiently interact on the same precursor polypeptide to yield cyclic peptides or splicing intermediates are preferred. Examples of non-naturally occurring split inteins from which such nucleic acid molecules can be derived include Psp Pol- 1 (Southworth, M.W., et al, *The EMBO J.* 17:918, 1998), Mycobacterium tuberculosis RecA intein, (Lew, B.M., *et al. J. Biol. Chem.* 273:15887, 1998; Shingledecker, K., *et al, Gene* 207:187, 1998; Mills, K.V., *et al. Proc. Natl. Acad. Sci.* USA 95:3543, 1998), Ssp DnaB/Mxe GyrA (Evans, T.C. *et al, J. Biol. Chem.* 274:18359, 1999), and Pfu (Otomo *et al, Biochemistry* 38:16040, 1999; Yamazaki *et al, J. Am. Chem. Soc.* 120:5591, 1998). (Emphasis added).

Applicants submit, that the specification teaches what the term "portion" of an intein means and that a person of ordinary skill in the art would understand the term "portion" that is, carboxy-terminal or amino-terminal ends of the protein.

The Examiner asserts that the term "derived from" renders the claims vague and indefinite. Applicants have amended the claims to remove reference to this term and amended the claims to use the term "corresponds to." Support for these amendments is found throughout the specification. See, for example, page 20, lines 11-15:

> Nucleotide sequences that encode the first portion of a split intein and the second portion of a split intein of the nucleic acid molecules within the invention can be derived from **known inteins.** A fairly comprehensive and descriptive list of such inteins is published by New England Biolabs at http//www.neb.conljinteins/ifltreghtml **Any of these known inteins can be used as long as they are compatible with invention.** (Emphasis added).

These amendments are deemed to overcome the Examiner's rejections. No new matter is added by virtue of these amendments and their entry is respectfully requested.

In view thereof, Applicants respectfully request reconsideration and withdrawal of the instant rejections.

The Examiner has rejected claims 1-11, 14-40, and 53-89 under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement. Applicants respectfully traverse.

Applicants describe in detail the invention and how the *in vivo* production and screening of cyclic peptide libraries are made and used and how these inteins function in the described reaction. The fact that these inteins can be from any genus reflects the versatility of the invention. Applicants provide details on selection of inteins, as discussed above. See, also for example, page 18, lines 5-23 through to page 19, lines 1-12:

> The **trans-splicing ability** of split inteins has been **exploited** to develop a general method of producing cyclic peptides and splicing intermediates displaying peptides in a **looped conformation**. In this method, a target peptide is interposed between **two portions of a split intein** in a precursor polypeptide. In an appropriate host system, the two portions of the split intein **physically come together to form an active intein** in a conformation that also forces the target peptide into a loop configuration. In this configuration, the ester isomer of the amino acid at the junction between one of the intein portions (e.g., I_N) and the target peptide is stabilized such that heteroatom from the other portion of the intein (e.g., I_C) can then react with the ester to form a cyclic ester intermediate. The active intein then catalyzes the formation of an aminosuccinimide that liberates a cyclized form of the target peptide (i.e., a lactone form), which then spontaneously rearranges to form the thermodynamically favored backbone cyclic peptide product (i.e., the lactam form). By arresting the reaction at given points before liberation of the cyclic peptide, splicing intermediates bearing the target peptide in a loop configuration can be produced. To produce such peptides, nucleic

acid molecules encoding a polypeptide having the target peptide sequence interposed between the two intein portions can be constructed. Introduction of these constructs into an expression vector provides a method for producing the polypeptide in a host system, where the polypeptide can be spliced into a cyclic peptide or a splicing intermediate. Using this method, several different cyclic peptides or splicing intermediates can be prepared to generate a library of cyclized or partially-cyclized peptides that can be screened for particular characteristics.

Referring to FIG. 1, an overview of an embodiment of the invention includes a method of making a cyclic peptide from a nucleic acid molecule. In this method, a nucleic acid molecule is prepared so that its nucleotide sequence encodes a polypeptide having in consecutive order a first portion of a split intein (e.g., I_C), a peptide to be cyclized (i.e., a target peptide), and a second portion of a split intein (e.g., I_N). The nucleic acid molecule can be incorporated into an expression vector to facilitate its expression in a host system where the nucleic acid can be transcribed and translated into a precursor polypeptide having the peptide to be cyclized interposed between the two split intein portions. By the steps described above, the two portions of the split intein come together and place the precursor peptide in a conformation that sets off chemical reactions that ultimately yield a cyclic peptide (see FIG. 2).

Applicants further describe, in detail, the invention and that inteins (RecA, DnaB, Psp, Pol-I or Pfu inteins) as claimed, are suitable in the methods of the invention. These inteins can be used to produce multiple split inteins. See, for example, page 22, lines 15-24 through to page 23, lines 1-20:

Nucleic Acid Molecules that Encode Multiple Split Inteins and Multiple Peptides

Using techniques similar to those described above, one skilled in the art could also prepare nucleic acid constructs that encode more than one set of two portions of a split intein interposed with peptides. For example, the invention includes nucleic acids molecules encoding a precursor polypeptide molecules comprised of N polypeptides (N = an integer greater than or equal to 1) and having N target peptides interposed between 2N intein portions such that any target peptide i (i= an integer greater than 1 representing the position of an target peptide in the precursor polypeptide) is interposed between intein portion 2i- 1 and 2i

> (e.g. target peptide 1 is between intein portion 1 & 2, target peptide 2 is between intein portions 3 & 4 etc.). As long as intein portions 2i-1 and 2i are not complementary (i.e. able to physically interact to catalyze a splicing event), target peptide i can not cyclize. If, however, intein portion 2i is complementary with intein portion 2i + 1 and intein portion 2N is complementary with intein portion 1, the entire ensemble of N polypeptides can perform N-1 trans splices (between 2 polypeptides) and 1 cis splice (ligating the two ends together) to give rise to a product wherein 1-N target peptides are covalently attached to one another in a cyclic peptide/protein (e.g., intein portions 2 & 3 trans-splice target peptides 1 & 2; intein portions four & five trans-splice target peptides 2 & 3; intein portions 2N-2 & 2N-1 transsplice target peptides N-i & N; and intein portions N & 1 cis-splice to close the cyclic product containing the N target sequences). The order of trans/cis splicing events is irrelevant. The slowest splicing species (whether it is the complementary intein portion 2N&1, 2&3 or 80&81) will by default perform the cis-splice.

Thus, nucleic acid constructs can be made that express two or more polypeptides each composed of a target peptide interposed between two portions of a split intein where the intein components are not complementary (i.e., do not derive from the same intein or otherwise cooperate to catalyze any of the cyclization reactions). In such constructs, no one polypeptide could be cyclized unless it was expressed in the presence of a second polypeptide having the appropriate complementary intein component. Constructs of such nucleic acids within the invention could encode only one polypeptide per construct or more than one polypeptide per construct (e.g., a bi-functional plasmid).

Examiner alleges that Applicants "do not define any specific nucleotide sequence and structure that is common to the members of each claimed genus." Applicants respectfully traverse. The sequences of these inteins are known and the common structural feature which is inherent of each intein is the ability to be cleaved and reassemble. See, for example, page 20, lines 11-24 through to page 21, lines 1-13:

> Nucleotide sequences that encode the **first portion** of a split intein and the **second portion** of a split intein of the nucleic acid molecules within the invention can be derived from **known inteins**. A fairly

comprehensive and descriptive list of such inteins is published by New England Biolabs at http//www.neb.conljinteins/ifltreghtml Any of these known inteins can be used as long as they are compatible with invention.

Nucleotide sequences that encode either naturally-occurring or artificially-produced split inteins can be used to generate the intein portions of nucleic acid molecules within the invention. Naturally-occurring split inteins are expressed in nature as two separate components that bind one another to form one active splicing agent. The nucleic acid molecules encoding these naturally-occurring components can thus be used in the invention. One example of a naturally-occurring split intein that may be used is Ssp DnaE (Wu et al. Proc. Natl. Acad. Sci. USA 95:9226,1998).

Inteins that are not split in their natural state (i.e., those that exist as one continuous chain of amino acids) can be artificially split using known techniques. For example, two or more nucleic acid molecules encoding different portions of such inteins can be made so that their expression yields two or more artificially split intein components. See, e.g., Evans *et al, J. Btol. Chem.* 274:18359, 1999; Mills *et al, Proc. Natl. Acad. Sci.* USA 95:3543, 1998. The nucleic acids that encode such non-naturally occurring intein **components (portions)** can be used in the invention. Those nucleic acid molecules that encode non-naturally occurring split intein portions which efficiently interact on the same precursor polypeptide to yield cyclic peptides or splicing intermediates are preferred. Examples of non-naturally occurring split inteins from which such nucleic acid molecules can be derived include Psp Pol- 1 (Southworth, M.W., et al, *The EMBO J.* 17:918, 1998), Mycobacterium tuberculosis RecA intein, (Lew, B.M., *et al. J. Biol. Chem.* 273:15887, 1998; Shingledecker, K., *et al, Gene* 207:187, 1998; Mills, K.V., *et al. Proc. Natl. Acad. Sci.* USA 95:3543, 1998), Ssp DnaB/Mxe GyrA (Evans, T.C. *et al, J. Biol. Chem.* 274:18359, 1999), and Pfu (Otomo *et al, Biochemistry* 38:16040, 1999; Yamazaki *et al, J. Am. Chem. Soc.* 120:5591, 1998). (Emphasis added).

Indeed, knowledge of inteins in the art, is such that the mere recitation of the word "intein" immediately conjures a genus of functionally equivalent protein sequences in the mind of the person of skill in the art which, when provided with the teachings of the present disclosure, readily allows the artisan to make and use the claimed invention. Applicants have also discussed the potential mechanism on page 5, beginning on line 3, through to page 6, lines 1-8.

In view of the foregoing, Applicants submit that claims 1-11, 14-40 and 53-89 satisfy 35 U.S.C. § 122, first paragraph, and as such, are allowable. Applicants respectfully request reconsideration and withdrawal of the instant rejection.

Claim Rejections Under 35 U.S.C. § 102

Claims 1-11 are rejected under 35 U.S.C. § 102(b) as being anticipated by Holford *et al.* (*Structure*. 1998 Aug 15; 6(8):951-6; PTO 1449 filed 6/15/2001).

Applicants respectfully traverse.

Holford *et al,* does not teach or disclose the use of N-terminal and a C-terminal end of an intein as taught by applicants. Holford *et al.* speculate on how head-to-tail cyclized recombinant peptides and proteins could be made using the taught Expressed Protein Ligation (EPL), where introduction of an N-terminal cysteine and a C-terminal thioester within the same polypeptide chain allows for **intramolecular native chemical** ligation; and that this process has been used to prepare synthetic circular protein domain (see entire document, especially p. 955, penultimate paragraph). A thioesterified protein, **required** for expressed protein ligation, as discussed by Holford, is **only possible** in a **synthetic** milieu and would not be recognized by the skilled artisan as being equivalent to an N- or C-terminal portion of an intein.

The Examiner asserts on page 5, second paragraph of the Office Action:

> The specification defines the word "intein" is a polypeptide sequence that can **catalyze a splicing reaction during post-translational processing** of a protein (see p. 13, lines 3-5). Thus, when the teachings Holford *et al.* are read in view of this definition of "intein", the N-terminal polypeptide sequence of the recombinant protein containing the introduced N-terminal cysteine is deemed to be the first portion of a split intein; the C-terminal polypeptide sequence containing the introduced C-terminal thioester is deemed to be the second portion of a split intein; and

> the polypeptide sequence in between is deemed to be the target peptide that is to be cyclized.

Applicants respectfully traverse. Holford *et al.*, **requires** the introduction of a cysteine and a thioester. See, for example, page 955, first column, last line of the second paragraph:

> It is also worth noting that introduction of both an N-terminal **cysteine** and a C-terminal **thioester** within the same polypeptide chain allows intramolecular native chemical ligation reactions to be performed, a process which has been used to prepare a synthetic circular protein domain. It is easy to conceive how head-to-tail cyclized recombinant peptides and proteins could be obtained in an analogous manner using EPL. (Citations omitted; Emphasis added).

Holford *et al.*, do not teach an intein as taught by applicants. Rather, Holford *et al.*, introduce an N-terminal cysteine and a C-terminal thioester which allows for an intra molecular ligation. These two **residues** are not inteins, but rather substitutions.

On page 5, lines 3-12, Applicants describe the method for producing and screening of cyclic peptide libraries *in vivo* using inteins which are neither taught or disclosed by Holford et al:

> A general method for the *in vivo* production and screening of cyclic peptide libraries has been discovered. In this method, a nucleic acid molecule is constructed such that a nucleotide sequence encoding the peptide to be cyclized is flanked on one end with a nucleotide sequence encoding the **carboxy-terminal portion** of a split (or trans) intein (C-intein or I_C) and on its other end with a nucleotide sequence encoding the **amino-terminal portion** of a split intein (N-intein or I_N). Expression of the construct within a host system such as a bacterium or eukaryotic cell results in the production of a fusion protein. The two split intein components (i.e., I_C and I_N) of the fusion protein then assemble to form an active enzyme that splices the amino and carboxy termini together to generate a backbone cyclic peptide. (Emphasis added).

Applicants teach that a first portion and a second portion is an N-terminal portion of a split intein and a carboxy-terminal portion of a split intein wherein each portion alone has no activity and requires the assembly of these portions to form an active enzyme. The number of amino acids that comprise each portion is not important, but the fact that assembly of each portion must form an **active** enzyme. Applicants further teach examples of split inteins. See, for example, page 6, lines 16-19:

> Both the **first portion** of a split intein and the **second portion** of a split intein can be derived from a naturally-occurring split intein such as Ssp DnaE. In other variations, one or both of split intein portions can be derived from non-naturally occurring split inteins such as those derived from RecA, DnaB, Psp Pol-I, and Pfu inteins. (Emphasis added).

Applicants teach that the N- and C-terminal inteins are **proteins that actually associate** to form a complex that initiates and drives the cyclization reaction. (See for example, page 18, lines 5-23 through to page 2, lines 1-12 and figures 1 and 2). Within this complex the cyclization reaction occurs with the **concomitant loss of the N- and C-terminal inteins**. Holford *et al.*, **do not teach** or suggest how to circularize a peptide molecule. Furthermore, Holford *et al.*, do not teach "a nucleic acid molecule encoding a polypeptide comprising a **first portion of a split intein**, a **second portion of a split intein**, and a target peptide interposed between the first portion of a split intein and a second portion of a split intein."

Applicants submit that Holford *et al.*, does not anticipate each and every limitation of claims 1-11 and as such claims 1-11 are allowable over Holford *et al.* In view thereof, Applicants respectfully request reconsideration and withdrawal of the instant rejection.

Claim Rejections Under 35 U.S.C. § 103

Claims 14-40 and 53-89 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Guan et al. (U.S. Patent 5,643,758) in view of Holford *et al.* (*Structure*. 1998 Aug 15; 6(8):951-6; PTO 1449 filed 6/15/2001).

As discussed above, Holford *et al.*, do not teach an intein as taught by applicants. Rather, Holford *et al.*, introduce an N-terminal cysteine and a C-terminal thioester which allows for an intra molecular ligation. These two **residues** are not inteins, but rather substitutions. Holford requires synthetic processing to create a thioesterified protein, and Holford neither teaches nor suggests a polypeptide, as taught by Applicants, that would be amenable to vectors and cells **and** function as N- and C-terminal inteins.

Guan *et al.*, do not teach how to make and purify cyclic peptides using inteins. Furthermore, Guan *et al.*, do not teach the "expression of the nucleic acid molecule in a host system produces the **polypeptide** in a form selected from the group consisting of: (a) a polypeptide that **spontaneously** splices **in the host system** to yield a cyclized form of the target peptide, and (b) a **splicing intermediate** of a **cyclized form** of the target peptide." Guan *et al.*, in fact, teaches away from using inteins as the purification method in Guan *et al.*, requires the use of a linking sequence such that use of blood coagulation Factor Xa is required. See Guan *et al.*, column 6, lines 43-64:

> A DNA fragment coding for a predetermined peptide may be employed to link the DNA fragments coding for the binding protein and protein molecule. The predetermined peptide is preferably one **which recognized and cleaved** by a **proteolytic agent** such that it cuts the hybrid polypeptide at or near the protein molecule without interfering with the biological activity of the protein molecule. One such DNA fragment coding for a predetermined polypeptide is described in Nagai et al., Nature, Vol. 309., pp. 810-812 (1984), the disclosure of which is hereby incorporated by reference. This DNA fragment has the oligonucleotide sequence: ATCGAGGGTAGG and codes for the polypeptide Ile-Glu-Gly-Arg. This polypeptide is cleaved at the carboxy side of the arginine residue using blood coagulation factor Xa. As noted above the **linking sequence**, in addition to providing a convenient cut site, may also serve as a polylinker, i.e. by providing **multiple restriction sites** to facilitate fusion of the DNA fragments coding for the target and binding proteins, and/or as a spacing means which separates the target and binding protein

which, for example, allows access by the proteolytic agent to cleave the hybrid polypeptide. (Emphasis added).

Guan *et al.*, do not teach or disclose use of inteins for the purification of a peptide that has been generated by the methods of the invention; nor the production of a polypeptide that **spontaneously** splices **in the host system** to yield a cyclized form of the target peptide, and (b) a **splicing intermediate** of a **cyclized form** of the target peptide. Furthermore, Guan *et al.*, require the use of an **external** agent to cleave the linker molecule. It would not be obvious to one of skill in the art to use Guan *et al.*, in view of Holford et al., to arrive at a method of purifying a cyclic peptide as taught by applicants. In fact Guan *et al.*, teach away from use of inteins.

In view of the foregoing, claims 14-40 and 53-89 are allowable under 35 U.S.C. § 103(a) over the cited references. Applicants respectfully request reconsideration and withdrawal of the instant rejection.

Applicants have made every effort to present claims which distinguish over the cited art, and it is believed that all claims are now in condition for allowance. However, Applicants request that the Examiner call the undersigned (direct line 561-671-3666) if anything further is required by the Examiner prior to issuance of a Notice of Allowance for all claims.

CONCLUSION

Applicants respectfully request entry of the foregoing amendments and remarks and reconsideration and withdrawal of all rejections. It is respectfully submitted that this application with claims 1-11, 14-40 and 53-89, is in condition for allowance. If there are any remaining issues or the Examiner believes that a telephone conversation with the Applicants' attorney would be helpful in expediting prosecution of this application, the Examiner is invited to call the undersigned at telephone number shown below.

Although, Applicants believe that no further extensions of time (beyond the one month petition) are required with submission of this paper, Applicants request that this submission also be considered as a petition for any extension of time if necessary. The Commissioner for Patents and Trademarks is hereby authorized to charge the amount due for any retroactive extensions of time and any deficiency in any fees due with the filing of this paper or credit any overpayment in any fees paid on the filing or during prosecution of this application to Deposit Account No. 50-0951.

Respectfully submitted,

AKERMAN SENTERFITT

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Docket No. 6818-18-1